REMARKS

Pending Claims

Claims 4 to 9 have been examined. New claims 10 and 15 have been added. Support for claims 10 and 11 can be found, for example, on page 13, lines10 to 26. Support for claims 12 to 15 can be found, for example, on page 6, lines 17 to 26; and page 9, lines 21 to 22. No new matter has been added. Thus, pending claims are claims 4 to 15.

Claim Rejections – 35 USC §101

Claims 4 to 9 have been rejected because the claimed invention is allegedly directed to non-statutory subject matter. The claims as amended are now directed to "a genomic DNA data containing medium." Support for this amendment can be found, for example, on page 10, line 14 to page 12, lines 3. No new matter has been added. The claimed invention is thus directed to statutory subject matter.

Furthermore, claims 5, 7, and 9 have been amended to depend on claim 4, the utility of which is clearly set forth on page 2, lines 13 to 18: the claimed invention is used to analyze genomic DNA. Thus, withdrawal of these rejections is respectfully requested.

Claim Rejections – 35 USC §112

Claims 4 to 9 have been rejected as not being described in the specification in such a way to reasonably convey to one skilled in the relevant art that the inventors had possession of the claimed invention. It is alleged that what comprises "pattern" in the claim is indefinite. To obviate this rejection, the amended claims are now directed to a medium containing genomic DNA data derived from a method as defined in the claims. Support for this amendment can be found, for example, on page 10, line 14 to page 12, lines 3. No new matter has been added.

Claims 5, 7, and 9 have been rejected over the recitation "wherein the pattern is one represented by Figs. 4, 5, 6, or 8" and for being directed to "figures." These claims have been amended to eliminate the references to "pattern" and "figures."

Claims 6 to 9 also have been rejected for depending on canceled claims. Claims 6 to 9 have been amended to depend on claim 4, directly or indirectly.

For the foregoing reasons, withdrawal of the §112 rejections of claims 4 to 9 is respectfully requested.

Claim Rejections – 35 USC §103

Claims 4 and 5 have been rejected as being unpatentable over Hatada et al. in view of Deugau et al. Applicants respectfully submit that the claims 4 and 5 would not have been obvious to a person of ordinary skill in the art for the following reasons.

The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F.2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990). As the following arguments will show, there is no suggestion of desirability to combine Hatada et al.'s RLGS with Deugau et al.'s method.

Hatada et al.'s restriction landmark genomic scanning (RLGS) method employs "(i) direct end labeling of the genomic DNA digested with a restriction enzyme and (ii) high-resolution, two-dimensional electrophoresis." (Page 9523, first complete paragraph from the bottom of the first column.) That is, Hatada et al.'s method involves cleaving the entire genome with a rare restriction enzyme that does not contain cytosine residues or is insensitive to methylation (see page 9524, first complete paragraph from the bottom of the second column) and labeling all the cleaved ends at both ends of each fragment (see Fig. 1) without any adapters attached to the ends. All the fragments are labeled. RLGS is introduced as an alternative to Southern blotting, which requires many repetitions scanning with probes for analyzing large genomes. (See page 9523, second complete paragraphs from the bottom of the first column.) Hatada et al. also cites that bands (spots) corresponding to loci could not be resolved by Southern blotting. RLGS, on the other hand, can simultaneously resolve thousands of spots derived from restriction cites and locate landmarks on mammalian genomes at intervals averaging about 1 megabase pair. (See page 9523, first complete paragraphs from the bottom of the first column.) Clearly, Hatada et al.'s RLGS is designed to replace the piece-wise, recursive approach of Southern blotting to analyze a large genome all at once. The thrust of the RLGS approach is to

avoid manipulating subsets of fragments of a large genome but to resolve the resulting fragments of a large genome all at once in a simple but powerful way.

Deugau et al., on the other hand, states that "selective attachment of indexing linkers having known base sequences in their cohesive ends to a subset of fragments bearing the complementary cohesive ends can be used for the detection identification, isolation, amplification, and manipulation of the subset of fragments." (Column 6, lines 25 to 29; emphasis added.) Deugau et al.'s method is practiced in the context of three major techniques: molecular cloning (column 1, lines 51 to 67; and column 2, line 47 to column 3, line 5), polymerase chain reaction (column 2, lines 1 to 18; and column 3, lines 6 to 20), and restriction fragment length polymorphism (column 2, lines 19 to 41; and column 3, lines 21 to 37). That is, Deugau et al. proposes to extend and enhance those three major techniques by the method of "indexing" the subsets of fragments. Albeit, purported to impart more efficiency (see column 1, lines 43 to 46), the indexing of nucleic acid fragments still must be carried out in a piece-wise, recursive manner if a large genome is to be analyzed. This is precisely the approach Hatada et al's RLGS aims to avoid. If proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984).

Thus, it is submitted that a person of ordinary skill in the art would not have combined Hatada et al. and Deugau et al. for the reason that Hatada et al.'s RLGS is designed to analyze the genome by labeling all of the fragments without adapters and does not involve recursively labeling subsets of fragments, which is an approach espoused by Deugau et al. if a large genome is to be analyzed.

It is alleged in the office action that "Deugau et al. expressly motivates the use of their indexing linkers [in Hatada et al.'s RLGS] by suggesting that it would be a powerful extension of known methods of nucleic acid analysis 'to provide a method for subsetting complex mixtures of nucleic acid in a consistent and efficient manner, to provide a level of information intermediate between the relatively crude measure of size of restriction fragments, and the precision of partial or complete sequence determination (Col. 3, lines 45 to 50).'" However, the above quote is a misinterpretation of Deugau et al.'s cited passage. What the passage suggests is that the Deugau et al.'s invention would be a power extension to the three main techniques mentioned in previous

paragraphs: molecular cloning (column 1, lines 51 to 67; and column 2, line 47 to column 3, line 5), polymerase chain reaction (column 2, lines 1 to 18; and column 3, lines 6 to 20), and restriction fragment length polymorphism (column 2, lines 19 to 41; and column 3, lines 21 to 37). The passage does not say or imply that Deugau et al's invention would be a powerful extension of methods that would include Hatada et al's RLGS.

RLGS per se does not involve cloning or PCR. Of the three techniques, restriction fragment length polymorphism (RFLP) may seem most similar to Hatada et al.'s RLGS because both involve gel electrophoresis, but the two are inherently different from each other. In RFLP, the target fragments or groups of fragments are identified by hybridization with specific probes (column 2, lines 32 to 41). RLGS does not employ any hybridization steps; in fact, the authors tout the advantage of the method for not having to conduct hybridization: "[t]his method can be applied to all organisms because no DNA probes are needed, unlike hybridization methods..." (page 9256, the last sentence at the bottom of the first column). A person of ordinary skill in the art would perhaps be directed to modify the three main techniques as taught in the patent to Deugau et al. but not Hatada et al.'s RLGS. Thus, it is submitted that the cited passage does not provide any motivation for a person of ordinary skill in the art to modify the methods of Hatada et al to include the indexing linkers and restriction enzymes taught by Deugau et al.

Also, the motivation to combine is allegedly attributed to the passage, "the use of a comprehensive panel of indexing linkers provides a means for attaching specific functional modifications to selected subsets of a complex mixture of nucleic acid fragments, preferably DNA, and identifying the molecules so modified. Such a defined subset of molecules may be further resolved by additional cleavage and indexing, or by any of the established techniques such as cloning, PCR amplification, gel electrophoresis, etc. (Col. 8, lines 25 to 32)." Again, it is asserted that Hatada et al.'s RLGS involves labeling all of the ends of the fragments without adapters and analyzing the resultant genome fragments all at once. On the other hand, Deugau et al., as the passage states, teaches labeling or modifying selective subsets of the fragments by using the indexing linkers and then suggests using established techniques such as cloning, PCR, or gel electrophoresis (i.e. RFLP) to further resolve the labeled or modified fragments. Clearly, many repetitive and recursive runs are needed for other subsets of the fragments to analyze the whole genome. This would defeat the intended purpose of Hatada et al. Hatada et al.'s RLGS is

provided as an alternative solution to techniques that require many recursive runs. Incorporating the teaching of Deugau et al. into Hatada et al.'s RLGS would necessarily change the intended purpose of Hatada et al. If the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious. *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959). A person of ordinary skill in the art would not have found the necessary motivation in Deugau et al. to combine its teaching with Hatada et al. or vice versa.

It is important to note that, in Hatada et al., the nucleotides of the restriction cites would have to be known and well-defined because the interest is to label all of the ends of all the fragments generated. For example, it is explained in the legend of Fig. 2 that terminal deoxynucleotidyltransferase is used to label the overhang, AT-'3, produced by *Pac* I with [α-^{32}P]ddATP. It is noted that the enzymes specified by Hatada et al. such as *Pac* I, *Swa* I, *Mlu* I, *Not* I, *BssH* II, *Asc* I, and *Sse*8387 I all leave well-defined cleaved ends -- *Pac* I: AT-3' overhang; *Swa* I: blunt; *Mlu* I: 5'-CGCG overhang; *Not* I: 5'-GGCC overhang; *BssH* II: 5'-CGCG overhang; *Asc* I: 5'-CGCG overhang; and *Sse*8397 I: TGCA-3' overhang. (See, for example, www.promega.com.)

In contrast, Deugau et al. teaches,

> This invention may be used to index nucleic acid fragments, preferably those containing 3,4 and 5 base cohesive ends. The indexing linkers can be used in conjunction with Type IIS restriction endonucleases, that is enzymes which cleave DNA at locations outside of the recognition site and which generate cohesive ends. Examples of Type IIS restriction endonucleases are FokI, BbvI, HgaI, BspMI and SfaNI.
>
> The indexing linkers can also be used in conjunction with restriction endonucleases which recognize interrupted palindrome sequences and cut the DNA irrespective of the intervening sequences to produce cohesive ends. Examples of interrupted palindrome recognizing restriction endonucleases, are SfiI BglI, and BstXI. (Column 7, lines 34 to 47.)

The type IIS restriction endonucleases and restriction endonucleases which recognize interrupted palindrome sequences all produce random cleaved ends. For example, *Fok* I, *Bbv* I, *Sfa*N I, and *Bsp*M I produce a 5'-NNNN overhang, where N can be A, T, C, or G; *Hga* I produces a 5'-NNNNN overhang; *Sfi* I and *Bgl* I produce a NNN-3' overhang; and *Bst*X I produces a NNNN-3' overhang. (See www.promega.com.)

Deugau et al. clearly did not intend to use its restriction enzymes for a method such as Hatada et al.'s RLGS.

Furthermore, Hatada et al. teaches that the restriction enzyme must not contain cytosine residues or be insensitive to methylation:

> Restriction enzymes are sensitive to side-specific methylation. However, to use the spots as the signal of landmarks on the genome, the detection of restriction landmarks should not be affected by DNA modification, such as methylation. This problem can be overcome by using a restriction enzyme insensitive to methylation. For exampie, as the only known site for the methylation is position 5 of the cytosine residue in vertebrates (6), a rare cleaving enzyme not containing the cytosine residue, such as *Pac* I (TTAATTAA) or *Swa* I (ATTTAAAT), or enzymes insensitive to 5-methylcytosine should be used. (Page 9524, first complete paragraph from the bottom of the second column.)

Deugau et al. does not make any mention that the enzymes need to be methylation insensitive. If fact, some of the enzymes such as *Sfi* I and *Bgl* I are methylation sensitive. This is a further indication that a person of ordinary skill in the art would not have combined the cited prior art references.

For completeness, it should be mentioned that Hatada et al. and Deugau et al. individually do not teach or suggest the present invention as claimed in claim 4 and 5. Thus, for the foregoing reasons, it is submitted that claims 4 and 5 would not have been made obvious to a person of ordinary skill in the art by the cited prior art references. Withdrawal of the rejections against claims 4 and 5 are respectfully requested.

Claims 6 to 9, which depend on claim 4, would not have been obvious to a person of ordinary skill in the art at least for the same reasons as claim 4.

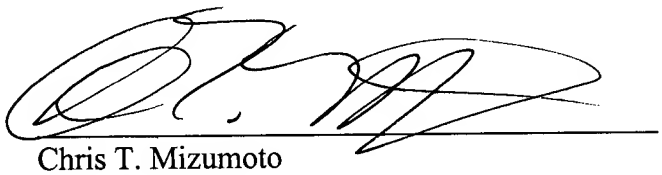
New Claims

Independent claims 10 and 12 are similar to claim 4. Thus, at least for the same reason as claim 4, claims 10 and 12 and their depend claims 11 and 13 to 15 would not have been obvious to a person of ordinary skill in the art.

Attached is a marked-up version of the changes being made by the current amendment.

Applicant asks that all claims be allowed. Enclosed is a check for the Petition for Extension of Time fee. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,



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Chris T. Mizumoto
Reg. No. 42,899

Fish & Richardson P.C.
45 Rockefeller Plaza, Suite 2800
New York, New York 10111
Telephone: (212) 765-5070
Facsimile: (212) 258-2291

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Version with markings to show changes made

In the claims:

Claim 4 has been amended as follows:

4. (Twice Amended) A genomic DNA [analytical pattern] data containing medium, which [has been] is obtained by means of a method of analysis comprising:

(a) treating genomic DNA with a first restriction enzyme that provides restriction enzyme cleavage sites of different sequences;

(b) linking one end of an adapter to a restriction enzyme cleavage site, which is cut by the first restriction enzyme and [that] is complementary to the end of the adapter, and labeling the other end of said adapter;

(c) treating the resulting DNA fragments with a second restriction enzyme to bring about first-dimensional fractionation;

(d) treating the fractionated DNA fragments of step (c) with a third restriction enzyme to bring about second-dimensional fractionation; and

(e) detecting the spots of the labeled DNA fragments fractionated in step (d).

5. (Amended) A [pattern] genomic DNA data containing medium according to claim 4, wherein [the pattern is one represented by Figs. 4, 5, 6, or 8] said adapter has a 3'-protruding end that is labeled by attaching two to ten radio- or fluorescent-labeled bases complementary to the 3'-protruding end.

6. (Amended) A genomic DNA [analytical pattern] data containing medium, which has been obtained by means of a method of analysis according to claim [2] 4, wherein the first restriction enzyme cuts the genomic DNA such that 3' end of the recognition site of the first restriction enzyme has a protruding sticky end.

7. (Amended) A [pattern] genomic DNA data containing medium according to claim [6] 4, wherein [the pattern is one represented by Figs. 4, 5, 6, or 8] the first restriction enzyme

comprises a recognition sequence that includes at least one N, where N can be any of A, G, C, or T and the linking end of the adapter having a ligating sequence is designed to anneal to the restriction enzyme cleavage site and has a base complementary to N in the recognition sequence of the first restriction enzyme.

8. (Amended) A genomic DNA [analytical pattern] data containing medium, which has been obtained by means of a method of analysis according to claim [3] 4, wherein the first restriction enzyme comprises BstXI, Bg1I, or MwoI.

9. (Amended) A [pattern] genomic DNA data containing medium according to claim 8, wherein [the pattern is one represented by Figs. 4, 5, 6, or 8] the medium comprises an electrophoresis gel.

10. (New) A genomic DNA data containing medium, which is obtained by a method comprising:

(a) treating genomic DNA with a first restriction enzyme that is sensitive to methylation of the genomic DNA;

(b) linking one end of an adapter to a restriction enzyme cleavage site, which is cut by the first restriction enzyme and is complementary to the end of said adapter, and labeling the other end of said adapter;

(c) treating the resulting DNA fragments with a second restriction enzyme to bring about first-dimensional fractionation;

(d) treating the fractionated DNA fragments of step (c) with a third restriction enzyme to bring about second-dimensional fractionation; and

(e) comparing the resulting spots of the labeled DNA fragments with a standard spot pattern derived from DNA fragments known not to be methylated.

11. (New) A genomic DNA data containing medium according to claim 10, wherein the first restriction enzyme comprises NotI, AccIII, or BssHII..

12. (New) A genomic DNA data containing medium, which is obtained by means of a method of analysis comprising:

(a) treating genomic DNA with a first restriction enzyme to produce restriction enzyme cleavage sites having a protruding 3' end;

(b) linking one end of an adapter to a restriction enzyme cleavage site, which is cut by the first restriction enzyme and is complementary to the end of the adapter, and labeling the other open end of said adapter;

(c) treating the resulting DNA fragments with a second restriction enzyme to bring about first-dimensional fractionation;

(d) treating the fractionated DNA fragments of step (c) with a third restriction enzyme to bring about second-dimensional fractionation; and

(e) detecting the spots of the labeled DNA fragments fractionated in step (d).

13. (New) A genomic DNA data containing medium according to claim 12, further comprising:

labeling the adapter comprises attaching labeled bases to the open end of the adapter.

14. (New) A genomic DNA data containing medium according to claim 12, wherein the adapter is labeled by attaching two to ten labeled bases to the open end of the adapter.

15. (New) A genomic DNA data containing medium according to claim 12, wherein the adapter is labeled by attaching two to ten radio- or fluorescent-labeled bases to the open end of the adapter.